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The magnetic susceptibilities of iron deposits in thalassaemic spleen tissue

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Abstract

The iron-specific magnetic susceptibility of tissue iron deposits is used in the field of non-invasive measurement of tissue iron concentrations. It has generally been assumed to be a constant for all tissue and disease types. The iron-specific magnetic susceptibilities χ_{Fe} for spleen tissue samples from 7 transfusion dependent β-thalassaemia (β-thal) patients and 11 non-transfusion dependent β-thalassaemia/Haemoglobin E (β/E) patients were measured at 37 °C. Both groups of patients were iron loaded with no significant difference in the distribution of spleen iron concentrations between the two groups. There was a significant difference between the mean χ_{Fe} of the spleen tissue from each group. The non-transfusion dependent β/E patients had a higher mean (±standard deviation) spleen χ_{Fe} ($1.55\pm0.23\times10^{-6}$ m^3/kg Fe) than the transfusion dependent β-thal patients ($1.16\pm0.25\times10^{-6}$ m^3/kg Fe). Correlations were observed between χ_{Fe} of the spleen tissue and the fraction of magnetic hyperfine split sextet in the ^{57}Fe Mössbauer spectra of the tissues at 78 K (Spearman rank order correlation $r=-0.54$, $p=0.03$) and between χ_{Fe} of the spleen tissue and the fraction of doublet in the spectra at 5 K ($r=0.58$, $p=0.02$) indicating that χ_{Fe} of the spleen tissue is related to the chemical speciation of the iron deposits in the tissue.

Keywords: Haemosiderin; Ferritin; Magnetic susceptibility; Thalassaemia; Iron

1. Introduction

Iron overload is a condition that affects patients with haemoglobinopathies who either require regular blood transfusions [1] or have increased dietary iron absorption, and also patients with various forms of hereditary hemochromatosis [2]. Measurement of tissue iron concentrations is a part of the clinical management of many of these patients [3]. Liver iron concentration (LIC) is considered to be a reliable indicator of overall body iron burden. However, direct measurement of LIC by chemical analysis of needle biopsy samples is problematic both because of the sampling error introduced by the small size of the sample obtained and because of the associated morbidity and mortality risks associated with biopsy [4–7]. Over the past two decades or so, non-invasive methods of measurement of LIC have been developed which exploit the large and positive magnetic susceptibility of tissue iron deposits relative to the small and negative magnetic susceptibility of the other components of body tissues [8–11]. The iron deposits in iron loaded tissue are predominantly in the form of haemosiderin and ferritin [12]. Haemosiderin is an insoluble pathological compound containing particles of hydrated iron(III) oxyhydroxide associated with organic components while ferritin is an iron storage and homeostasis protein [13]. Ferritin is composed of 24 polypeptide subunits that self assemble to form an approximately spherical shell-like structure with an external diameter of approximately 12 nm and an internal cavity diameter of approximately 8 nm [14]. Iron is stored within the cavity in the form of a particle of hydrated iron(III) oxyhydroxide. Although both ferritin and haemosiderin have an inorganic iron(III) oxyhydroxide component, the chemical speciation of this component can vary. Three different chemical species of tissue iron(III) oxyhydroxide have been identified namely (i) ferrihydrite (a poorly crystalline mineral with the approximate formula $5Fe_2O_3{\cdot}9H_2O$, (ii) a mineral with a structure related to very poorly crystalline goethite (goethite has the formula α-FeOOH),

Abbreviations: W–M–W, Wilcoxon–Mann–Whitney

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and (iii) a non crystalline iron(III) oxyhydroxide [13,15,16]. The chemical speciation of these iron(III) oxyhydroxides has been shown to be different in different identifiable groups of iron loaded patients [17]. Since different chemical speciation of iron oxides is known to lead to different iron-specific magnetic susceptibilities [18], a knowledge of the magnetic susceptibilities of ferritins and the different forms of haemosiderin is of practical importance in the field of non-invasive measurement of tissue iron concentrations using methods such as biomagnetic susceptometry and magnetic resonance imaging that exploit the relatively large magnetic susceptibility of these materials.

The magnetic susceptibilities of ferritin and haemosiderin were first measured by Michaelis and coworkers [19]. They made measurements on samples of horse spleen ferritin at 27 °C and haemosiderin at 22 °C yielding iron specific magnetic susceptibility values of $1.33\ (\pm 0.02)\times 10^{-6}$ m^3 /kg_{Fe} for the ferritin and 1.1×10^{-6} m^3/kg_{Fe} for the haemosiderin. This work was later followed by measurements at 23 °C on human ferritin and haemosiderin isolated from liver tissue by Schoden and Sturgeon [20] yielding values of 1.6×10^{-6} m^3/kg_{Fe} for the ferritin and values of 1.5, 2.1, and 1.2×10^{-6} m^3/kg_{Fe} for three different human haemosiderin samples. The value of 1.6×10^{-6} m^3/kg_{Fe} for human ferritin measured by Schoden and Sturgeon has subsequently been used by Fischer and coworkers [21] to obtain non-invasive in vivo measurements of liver iron concentrations in iron loaded patients using the technique of magnetic biosusceptometry [22,23]. This value is now routinely used in biosusceptometry measurements to determine liver iron concentrations. However, the biological variability in the magnetic susceptibility measurements of Michaelis and coworkers [19] and Schoden and Sturgeon [20] appears to be larger than their reported analytical variability suggesting that the magnetic susceptibility of tissue iron deposits may vary between individuals. Furthermore, since the early measurements of the magnetic susceptibilities of ferritin and haemosiderin, there has been the discovery that there are different chemical species of iron(III) oxyhydroxide associated with different samples of haemosiderin and that the different chemical species have different magnetic properties at cryogenic temperatures [13,16,24]. These observations raise the questions of whether or not there are significant differences in the body temperature magnetic susceptibilities of the different chemical species of tissue iron deposit and to what degree these differences might affect measurements of tissue iron concentration by biomagnetic susceptometry techniques when different species are present or when the chemical speciation is unknown.

In order to address these questions, the iron specific magnetic susceptibilities of iron loaded spleen tissues from two identifiably different groups of iron-loaded patients have been measured. Spleen tissue was chosen because it is readily available after splenectomy procedures. The two groups of patients have been shown in a previous study to have different chemical speciation of the spleen tissue iron deposits [17]. The characterisation of the chemical speciation of the iron in these tissues is extended in this study.

2. Materials and methods

All human tissue samples were obtained under Human Ethics Committee guidelines (guidelines from human rights committees in both Australia and Thailand). Samples of spleen were obtained from two identifiably different groups of iron loaded patients, namely 7 β-thalassaemic patients who had received regular red cell transfusions and iron chelation therapy and 11 β-thalassaemia/haemoglobin E patients who had received few, if any, red cell transfusions and no iron chelation therapy. These tissue samples have been shown in a previous study to have different chemical speciation of tissue iron deposits with the β-thalassaemic tissues having a greater fraction of the iron in the form of goethite-like haemosiderin than the β-thalassaemia/Hb E samples [17]. The β-thalassaemic samples were obtained from patients who had undergone splenectomy at Princess Margaret Hospital, Perth. The ages of the patients at the time of splenectomy ranged from 10 to 15 years and all had received regular transfusions of packed red cells together with chelation therapy. Regular transfusions of packed red cells (15–20 ml/kg body mass/month) were started at about 12 months of age. This regimen is equivalent to a rate of approximately 0.5 units of packed red cells per month at age 1 year increasing to 1–2 units of packed cells per month by the age of approximately 10 years. Chelation therapy using desferrioxamine (Desferral, Ciba-Geigy) was started between the ages of 2 and 3 years. Samples of β-thalassaemia/Hb E spleen were obtained either post splenectomy or post mortem from 11 patients from Siriraj Hospital, Bangkok. The age of the patients ranged from 20 to 40 years and they had received few (less than a total of 30 units of packed red cells), if any, red cell transfusions and no chelation therapy. Tissue samples were freeze dried and ground to a powder prior to magnetic susceptometry measurements and iron content analysis with atomic absorption spectrometry.

A sample of human liver ferritin was also prepared to enable comparison of results with those on human liver ferritin measured by Schoden and Sturgeon [20]. The ferritin was isolated from four livers obtained post-mortem from β-thalassaemia/Hb E subjects according to the method of ferritin purification described by Chua-anusorn and St. Pierre [25]. These subjects had received few, if any, blood transfusions.

Mössbauer spectra were recorded in transmission geometry using a ^{57}Co in rhodium foil source. The source was driven at constant acceleration from −13 to +13 mm/s with a double ramp waveform. Spectra were subsequently folded to eliminate the parabolic background caused by variation in the solid angle subtended by the detector window about the source. The resulting spectra consisted of 250 data points with a background count of between approximately 1×10^6 and 2×10^6 per channel. The counting time was determined by the iron concentration of the sample under study. The velocity scale was calibrated with reference to the spectrum of an α-iron foil at room temperature, the centre of the spectrum being taken as zero velocity. Freeze dried samples of tissue were packed into 10 mm diameter Perspex sample holders for the 78 K spectra and 13 mm copper sample holders with Mylar windows for the 5 K measurements. The thickness of the sample was adjusted so that the 14.4 keV Mössbauer γ rays were attenuated by approximately $1/e$. Sample temperatures were maintained at 78 K or at 5 K during the spectral data acquisition using a liquid nitrogen or liquid helium cryostat respectively.

Each Mössbauer spectrum was initially fitted with a doublet and sextet of Lorentzian peaks using a sum of squares minimization procedure. In some spectra, a small signal owing to haem-iron was detectable and as such a second doublet was included in the fitting procedure. The doublet from haem-iron is clearly distinguishable from that of paramagnetic haemosiderin iron since it exhibits a much larger quadrupole splitting giving a clearly resolved peak even when superimposed on a paramagnetic haemosiderin doublet [26]. In such cases, the relative spectral area of the haem doublet was used to measure the fraction of haem-iron in the sample. The two peaks in each doublet were constrained to be of equal area and width while the ratio of areas of the outer to middle to inner pairs of peaks of the sextet were constrained to be in the ratio 3:2:1. The linewidths, centre shift, and quadrupole splitting of the doublet were allowed to vary freely during the fits as were the linewidths, magnetic-hyperfine-field splitting, centre shift, and quadrupole perturbation on the sextet. This fitting procedure yields reproducible spectral parameters and doublet to sextet area ratios for spectra with high signal to noise ratios. However, for some of the 78-K spectra with low sextet-signal to noise ratios, this fitting procedure tends to give unreliable results.

Mean values for the Mössbauer spectral parameters of the 78-K sextet signal in the 4 spleen spectra with the highest sextet-signal to noise ratio were used as a standard with which to refit all of the spleen tissue 78-K spectra. All of the spleen 78-K spectra were refitted with a high-spin Fe(III) doublet, a haem doublet where necessary, and the standard sextet. During the fitting procedure, the linewidths, magnetic-hyperfine-field splitting, centre shift, and quadrupole perturbation of the sextet were allowed to vary by up to one standard deviation from the mean values derived from the 4 spectra with the highest sextet-signal to noise ratios. The area of the sextet was allowed to vary freely. In this way the relative spectral area of the sextet in spectra with very low sextet-signal to noise ratios could be more reliably assessed assuming that the Mössbauer spectral parameters of the sextet do not vary significantly from sample to sample.

Samples of the freeze-dried powders (both tissues and ferritin sample) were compressed into pellets of approximate mass 60 mg for magnetic susceptometry measurements. All pellets were less than 4.5 mm in length. For three of the spleens, duplicate pellets were made in order to test reproducibility of measurements. Pellets were mounted in long uniform plastic tubes and were held in place in the tubes for magnetometry measurements with plastic collars of less than 8 mm in length that surrounded the pellets symmetrically. The tubes were much longer than the dimensions of the sense coils and hence do not contribute to the measured signal. However, the diamagnetic susceptibility of the collars was measured independently and accounted for in the measurement of the sample magnetic susceptibilities by subtraction of the moment of the collar from the moment of the collar and pellet.

Magnetic susceptometry measurements were made using a Quantum Design magnetic properties measurement system (MPMS-7). The instrument uses a sensor based on a superconducting quantum interference device (SQUID) for detection of sample magnetic moments as they are passed through a second-order gradiometer superconducting sensor coil system. The sensor coil system consists of a central coil with 2 counter-clockwise turns and 2 single clockwise turn coils 2 cm above and 2 cm below the central coil. As both collars and sample pellets were less than 10 mm in length they were small with respect to the coil spacing and hence were approximated as point dipoles.

Sample magnetic moments were measured as a function of applied magnetic field over the range of fields $\pm 2.4 \times 10^6$ A/m (±30 kOe). Sample temperatures were maintained at 37 °C during the measurements. At this temperature and over this magnetic field range the magnetic moment of the samples was approximately linear with applied magnetic field, consistent with previous observations of magnetic susceptibility of ferritin at temperatures close to body temperature [27,28]. Iron-specific magnetic susceptibilities were determined by measuring the slope of the mass-specific magnetic moment against applied magnetic field and correcting for collar magnetic susceptibility and iron-free-tissue magnetic susceptibility (see Results).

Tissue samples were stored in the freeze dried state between measurement of Mössbauer spectra and measurement of the magnetic susceptibility. In order to check that no change in chemical speciation had occurred during the intervening period, a Mössbauer spectrum was recorded for a second time at 78 K for a sample of spleen tissue with a mixture of spectral species after the magnetic susceptibility measurement. No changes in the Mössbauer spectral parameters were detectable indicating that there had been no detectable change in the chemical speciation of iron within the sample during the period of the study.

3. Results

Elemental analysis of the tissue samples indicated tissue iron concentrations ranging from 2.2 to 21 mg Fe g^{-1} dry tissue. The means (±standard deviation) of the non-haem iron concentration of the β-thalassaemic and β-thalassaemia/Hb E samples were 7.7 (±6.3) and 5.6 (±1.9) mg/g dry tissue, and the medians (and interquartile ranges) were 6.1 (3.6–8.9) and 5.7 (4.4–9.3) mg/g dry tissue respectively. There was no significant difference in the distribution of tissue iron concentrations between the two groups of patients as determined by a Wilcoxon–Mann–Whitney (W–M–W) test ($p = 0.93$).

3.1. Mössbauer Spectroscopy

Mössbauer spectra of the spleen tissue samples comprised a superposition of a doublet spectral component (attributed to paramagnetic or superparamagnetic high-spin iron(III)) and a sextet spectral component (attributed to magnetically blocked iron(III)) at both 78 K and 5 K (see Fig. 1). For an explanation of terms and physical principles related to the interpretation of the Mössbauer spectra of tissue iron deposits, see St Pierre et al. [13]. For some samples a small doublet attributable to haem iron was also apparent (1 spleen from a transfusion dependent β-thalassaemia patient showed a 20% haem doublet; 2 spleens from non-transfusion dependent β-thalassaemia/Hb E patients showed 6% and 1% haem doublets each; for all other samples a haem doublet was not detectable). Mean values for the fitted parameters of each spectral component are given in Table 1. The fraction F_S of the non-haem spectral signal in the form of a sextet at 78 K is related to the fraction of non-haem tissue iron in the form of the goethite-like iron oxyhydroxide [13,15,29]. The

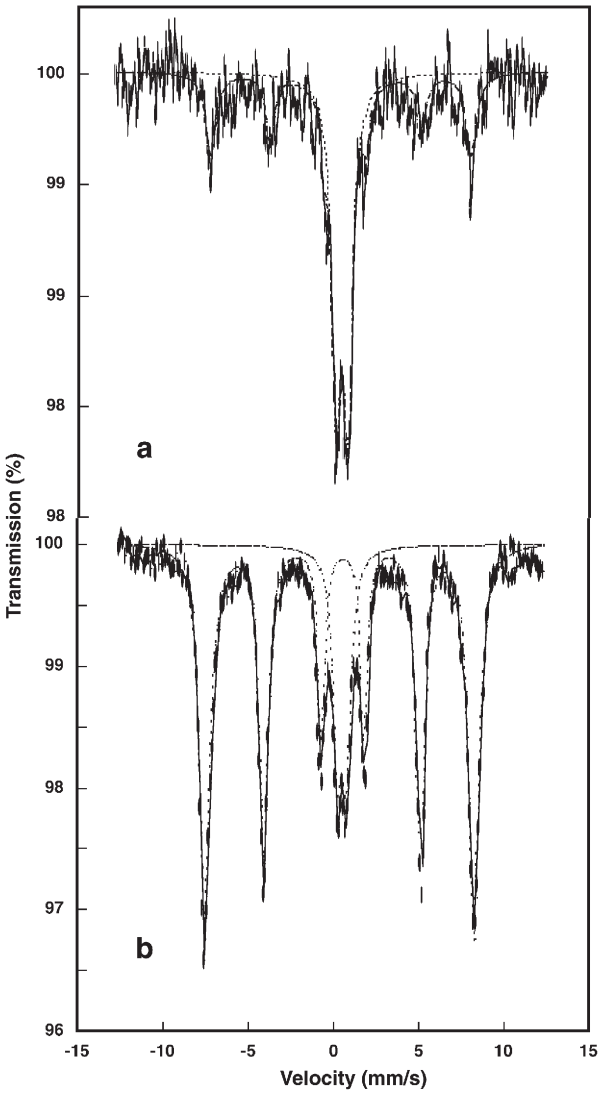

Fig. 1. Mössbauer spectra from a sample of β-thalassaemia/Hb E spleen tissue sample at (a) 78 K and (b) 5 K.

Table 1
Mean values and standard deviations (or ranges) for Mössbauer spectral parameters of doublet and sextet components in thalassaemic tissues at 78 K and at 5 K

	Spectral component	δ (mm/s)	ΔE_Q (mm/s)	B_{hf} (T)	Γ (mm/s)
78 K	Doublet	0.46±0.02	0.67±0.03		0.63±0.07
	Sextet	0.47 (0.42 to 0.51)	−0.21 (−0.28 to −0.13)	47.0 (46.5 to 47.4)	0.86 (0.73 to 0.99)
5 K	Doublet	0.50±0.02	0.55±0.04		0.56±0.08
	Sextet	0.47±0.04	−0.15±0.04	48.9±5.9	0.59±0.21

δ is the centre shift, ΔE_Q is the quadrupole splitting or quadrupole perturbation of the doublet and sextet component respectively, B_{hf} is the magnetic hyperfine-field splitting and Γ is the full linewidth at half-height. For the sextet component, Γ is measured from the outer peaks.

mean (±standard deviation) of F_S for spleens from the transfusion dependent β-thalassaemia patients was 0.27±0.12 and from the non-transfusion dependent β-thalassaemia/Hb E patients was 0.129±0.036. The medians (and interquartile ranges) of F_S were 0.26 (0.13–0.33) and 0.125 (0.102–0.243) for the β-thalassaemia and β-thalassaemia/Hb E patients respectively. The distributions of F_S for the two patient groups were significantly different (W–M–W test $p=0.01$) (Fig. 2). The fraction F_D of non-haem spectral signal in the form of a doublet at 5 K is reported to be related to the fraction of tissue non-haem-iron in the form of non-crystalline iron oxyhydroxide [13,15,29]. The mean (±standard deviation) of F_D for spleens from the transfusion dependent β-thalassaemia patients was 0.22±0.08 and from the non-transfusion dependent β-thalassaemia/Hb E patients was 0.43±0.19. The medians (and interquartile ranges) of F_D were 0.26 (0.18–0.36) and 0.41 (0.25–0.59) for the β-thalassaemia and β-thalassaemia/Hb E patients respectively. The distributions of F_D for the two patient groups were not found to be significantly different using a W–M–W test. A statistically significant correlation was found between F_S and tissue non-haem-iron concentration but not between F_D and tissue non-haem-iron concentration. A weak correlation was found between F_S and F_D (Spearman rank order correlation coefficient 0.47, $p=0.051$). The relationship between the two parameters appears non-linear (Fig. 3) with F_S remaining at approximately 0.10 until F_D drops below approximately 0.35. As F_D drops below 0.35 there is a very rapid rise in F_S to values around 0.40.

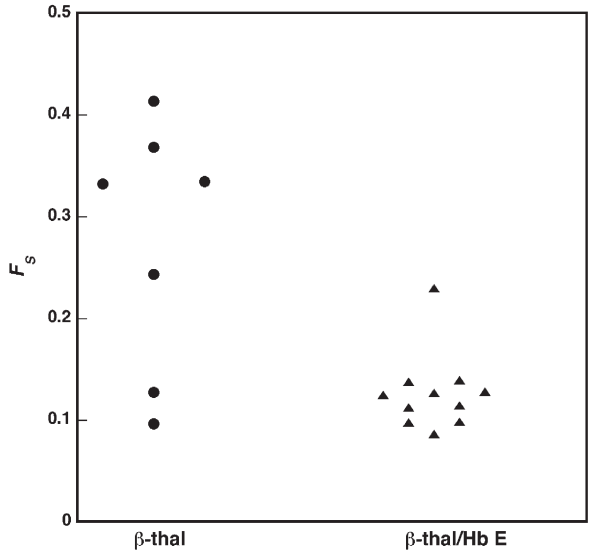

Fig. 2. Scatter plot of F_S, the fraction of Mössbauer spectrum recorded at 78 K in the form of a sextet for β-thalassaemia (●) and β-thalassaemia/Hb E (▲) spleen tissue samples.

3.2. Magnetic susceptibility measurements

The mass-specific magnetic susceptibility, χ_{Mass}, of each tissue sample is shown plotted against tissue non-haem-iron concentration in Fig. 4. The relationship between mass-specific magnetic susceptibility and tissue non-haem-iron concentration appears to be different for the two patient groups with the transfusion-dependent β-thalassaemia patients exhibiting a generally lower non-haem-iron-specific magnetic susceptibility, χ_{Fe}, than the non-transfusion-dependent β-thalassaemia/Hb E patients. The mass-specific magnetic susceptibility and non-haem-iron concentration data were fitted with the following model, based on Weidemann's mixture equation

$$\chi_{Mass} = \chi'_{Fe} \times [Fe] + \chi_{Tissue} \times (1 - [Fe]), \quad (1)$$

where χ_{Tissue} is the mass-specific magnetic susceptibility of non-haem-iron-free spleen tissue and [Fe] is the tissue non-haem-iron concentration expressed as the fraction of the mass of the tissue that is non-haem-iron. Fits of this model to the data from each

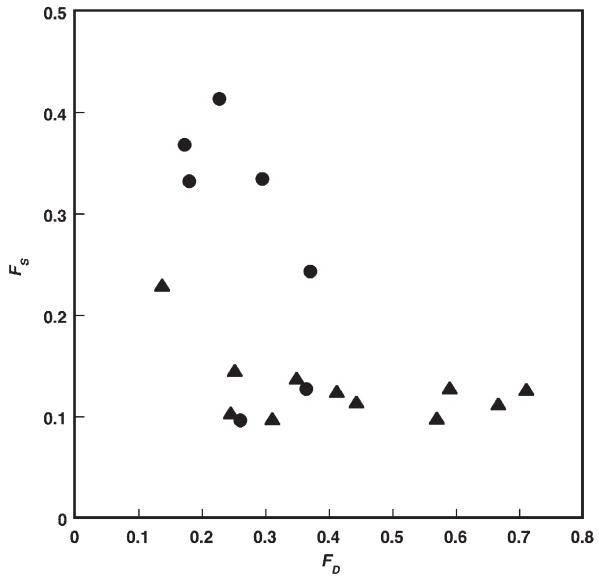

Fig. 3. Relationship between F_S measured at 78 K (related to the fraction of goethite-like iron oxyhydroxide in tissue) and F_D measured at 5 K (related to the fraction of non-crystalline iron oxyhydroxide in tissue) for β-thalassaemia (●) and β-thalassaemia/Hb E (▲) spleen samples.

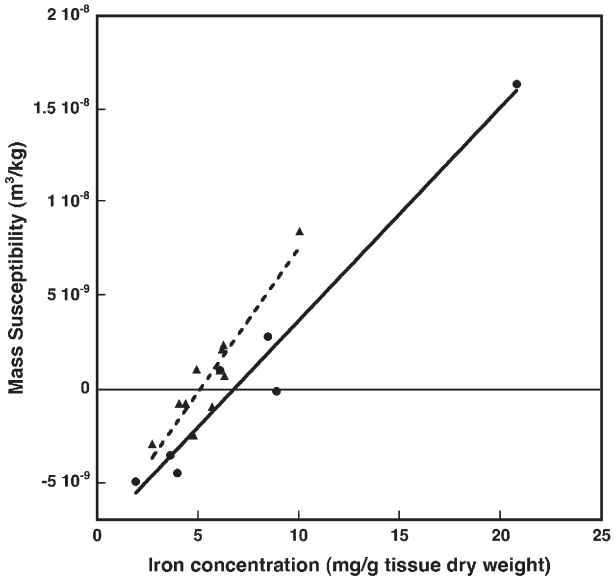

Fig. 4. Mass susceptibility of β-thalassaemia (●) and β-thalassaemia/Hb E (▲) spleen tissue samples versus iron concentration.

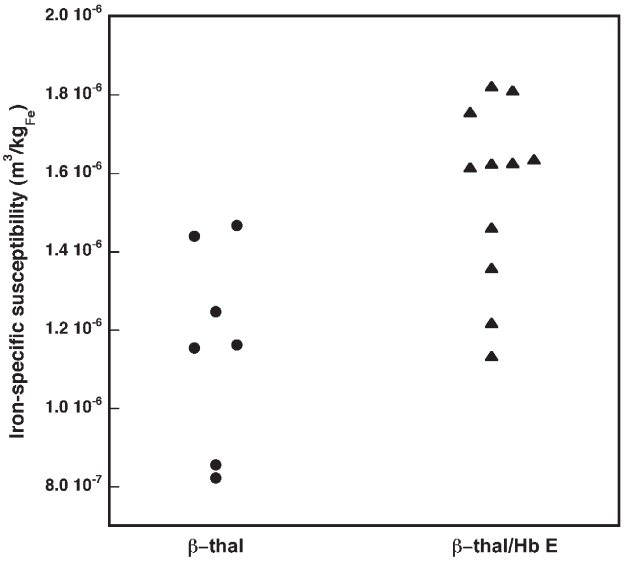

Fig. 5. Distribution of iron-specific magnetic susceptibilities of β-thalassaemia (●) and β-thalassaemia/Hb E (▲) spleen tissue samples (p-value of 0.01).

patient group are shown in Fig. 4 yielding average non-haem-iron-specific magnetic susceptibilities (χ'_{Fe}) of $1.10\pm0.10\times10^{-6}$ m^3/kg_{Fe} and $1.60\pm0.20\times10^{-6}$ m^3/kg_{Fe} and mass-specific magnetic susceptibilities for non-haem-iron-free tissue (χ_{Tissue}) of $-7.8\pm1.0\times10^{-9}$ m^3/kg dry tissue and $-7.9\pm1.3\times10^{-9}$ m^3/kg dry tissue for the transfusion-dependent β-thalassaemia and non-transfusion-dependent β-thalassaemia/Hb E patients respectively. It should be noted that the values of the average non-haem-iron-specific magnetic susceptibilities deduced from this model are based on the assumption that there is no increase in concentration of other high magnetic susceptibility elements concomitant with the non-haem-iron concentration increases. The validity of this assumption is supported by a previous study of macro, trace, and ultra-trace elements in thalassaemic tissues [30].

The values for the mass-specific magnetic susceptibilities of non-haem-iron-free tissue (χ_{Tissue}) from the above analysis were used to calculate a value of the non-haem-iron-specific magnetic susceptibility (χ_{Fe}) for each individual tissue specimen using the following equation

$$\chi_{Fe} = \chi_{Tissue} + \frac{\chi_{Mass} - \chi_{Tissue}}{[Fe]} \quad (2)$$

The distributions of values of χ_{Fe} for each patient group are shown in Fig. 5. The mean (±standard deviation) of χ_{Fe} for spleens from the transfusion-dependent β-thalassaemia patients was $1.16\pm0.25\times10^{-6}$ m^3/kg_{Fe} and from the non-transfusion dependent β-thalassaemia/Hb E patients was $1.55\pm0.23\times10^{-6}$ m^3/kg_{Fe}. The medians (and interquartile ranges) of χ_{Fe} were 1.16 $(0.85–1.44)\times10^{-6}$ m^3/kg_{Fe} and 1.63 $(1.36–1.75)\times10^{-6}$ m^3/kg_{Fe} for the β-thalassaemia and β-thalassaemia/Hb E patients respectively. The distributions of χ_{Fe} for the two patient groups were significantly different (W–M–W test $p=0.01$). A statistically significant correlation was observed between χ_{Fe} and tissue non-haem-iron concentration neither for each patient group alone nor for the two groups combined.

Fig. 6 shows the values of χ_{Fe} for each tissue specimen plotted against F_S. There is a statistically significant correlation between χ_{Fe} and F_S (Spearman rank order correlation coefficient −0.54, $p=0.03$) with higher values of F_S yielding generally lower values of χ_{Fe}. Fig. 7 shows the values of χ_{Fe} for each tissue specimen plotted against F_D. There is a statistically significant correlation between χ_{Fe} and F_D (Spearman rank order correlation coefficient 0.58, $p=0.02$) with higher values of F_D yielding generally higher values of χ_{Fe}.

The iron-specific magnetic susceptibility of the pooled human liver ferritin sample was measured by assuming that

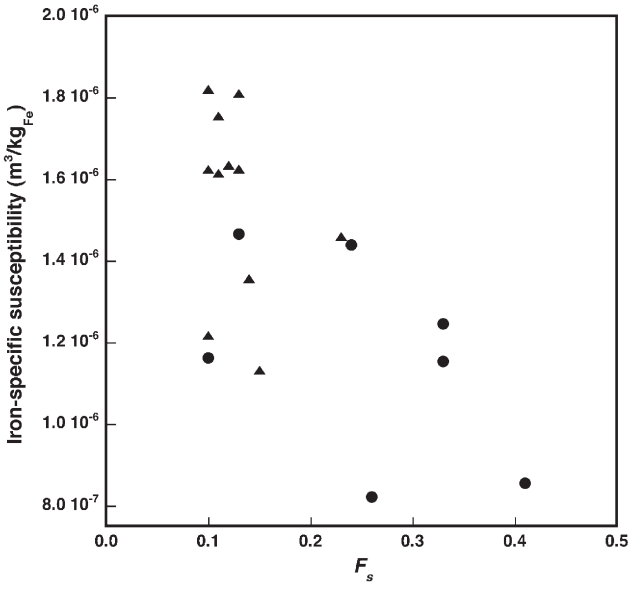

Fig. 6. Iron-specific magnetic susceptibility of β-thalassaemia (●) and β-thalassaemia/Hb E (▲) spleen tissue samples versus non-haem F_S measured at 78 K. (Spearman rank order correlation p-value 0.03).

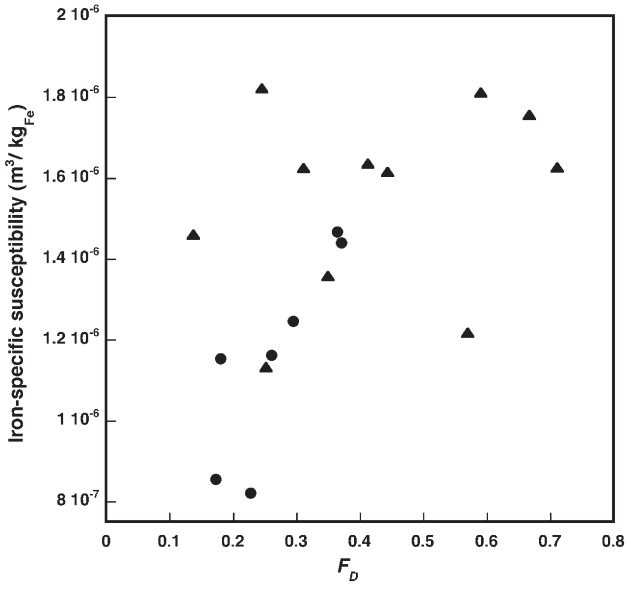

Fig. 7. Iron-specific magnetic susceptibility of β-thalassaemia (●) and β-thalassaemia/Hb E (▲) spleen tissue samples versus F_D. (Spearman rank order correlation p-value 0.02).

the mass-specific magnetic susceptibility of the non-iron component of ferritin is equivalent to the average mass-specific magnetic susceptibilities of non-haem-iron-free spleen tissues (χ_{Tissue}) -7.8×10^{-9} m^3 kg. It is worth noting that even a reasonably large percentage error on the value of the mass-specific magnetic susceptibility of the non-iron component of ferritin has very little effect on the result obtained for the iron-specific magnetic susceptibility of ferritin. The value of χ_{Fe} for the ferritin sample was found to be 1.14 $(\pm0.11)\times10^{-6}$ m^3 kg Fe^{-1} which is consistent with the value of 1.33 $(\pm0.02)\times10^{-6}$ m^3 kg Fe^{-1} obtained for horse spleen ferritin [19] but is significantly less than the value of 1.64 $(\pm0.04)\times10^{-6}$ m^3 kg Fe^{-1} obtained by Shoden and Sturgeon [20] for human liver ferritin.

Measurement of χ_{Fe} for three duplicate samples of homogenised spleen tissue indicated an overall analytic variability of approximately 9.6%.

4. Discussion

The spleen samples from the two groups of patients covered a range of tissue non-haem-iron concentrations but the distributions of non-haem-iron concentration in the two groups were similar thus enabling comparisons to be made between the two groups. In all cases the predominant form of iron in the tissues was non-haem iron.

The Mössbauer spectra recorded for all of the tissues in this study consisted of a central doublet characteristic of high-spin Fe(III) superposed on a spectral sextet, the sextet being due to magnetic-hyperfine-field splitting of the ^{57}Fe nuclear energy levels. Similar central doublet and sextet signals have been observed in previous reports of Mössbauer spectra of human tissues or extracts from human tissues [13,16,29,31–43]. On the basis of these previous reports these spectral components can be identified as being due to polynuclear iron(III) oxyhydroxide deposits in the tissue. The assignment of these signals to polynuclear iron(III) oxyhydroxide deposits is consistent with the fact that the predominant form of iron found in iron loaded tissues is usually haemosiderin [44]. The doublet component observed in the spectrum at 78 K has parameters consistent with (a) ferritin, (b) non-crystalline haemosiderin, (c) haemosiderin based on the structure of the mineral ferrihydrite, or (d) the doublet component associated with haemosiderin based on the structure of the mineral goethite (the doublet component being due to those haemosiderin particles with a smaller magnetic anisotropy energy) [13,15,16]. As such it is not possible to unambiguously identify the source of this signal and the signal may be due to a combination of the above forms of iron. However, the sextet component in the spectrum at 78 K can be identified as being due to the presence of haemosiderin based on the structure of the mineral goethite since this is the only form of tissue iron deposit known to give a Mössbauer spectral sextet component with these parameters at 78 K [33,34,45]. Similarly, while the sextet component at 5 K can be associated with any or all of ferritin, ferrihydrite based haemosiderin, and goethite-like haemosiderin, the doublet component observed at 5 K can be uniquely assigned to non-crystalline iron oxyhydroxide [13,15,16]. However, it is possible to rank the different forms of haemosiderin, goethite-like, ferrihydrite-like and non-crystalline iron oxyhydroxide, in order of decreasing crystallinity.

Hence, as discussed elsewhere [17], the significantly different distributions of F_S for the two groups of patients indicate that the chemical speciation of iron is different between the two groups with the transfusion dependent β-thalassaemia patients having a greater fraction of their spleen iron in the form of the goethite-like iron oxyhydroxide. In this study it has been shown that the distributions of F_D for the two groups of patients are not significantly different indicating that the fraction of spleen iron in the form of non-crystalline iron oxyhydroxide is not significantly different for the two groups of patients.

The observed negative correlation and apparently non-linear relationship between F_S and F_D suggests that pathological conditions that favour the formation of goethite-like haemosiderin in spleen tissue hinder the formation of non-crystalline iron oxyhydroxide deposits and vice versa.

The observed linear relationships between the mass-specific magnetic susceptibility and tissue non-haem-iron concentration (Fig. 4) confirm that magnetic susceptometry techniques can be used to assess tissue non-haem-iron concentrations. However, the fact that the chemical speciation of the non-haem-iron appears to determine the non-haem-iron-specific magnetic susceptibility of the tissue (Figs. 6 and 7) suggests that errors in estimations of tissue non-haem-iron concentrations will in some part be due to the biological variability of the chemical speciation between different individuals. A likely explanation for the negative correlation between χ_{Fe} and F_S (Fig. 6) is that the goethite-like deposits associated with the sextet signal in the Mössbauer spectra at 78 K are more crystalline (as determined by electron diffraction measurements) and have larger particle sizes than the other forms of iron(III) oxyhydroxide tissue deposits [25]. As such, there is likely to be a greater degree of

spin compensation in the antiferromagnetic structures thus reducing χ_{Fe}. Similar arguments can be made to explain the positive correlation between χ_{Fe} and F_D (Fig. 7). The doublet component in the Mössbauer spectra at 5 K is associated with non-crystalline iron(III) oxyhydroxide tissue deposits [25] and hence these deposits are expected to have a greater fraction of uncompensated spins in their structures thus increasing χ_{Fe}.

The data presented in this report are for spleen tissue. However, the organ of primary interest in the clinical use of biomagnetic susceptometry is the liver. It is currently unknown whether there is a similar magnitude of variation in the chemical speciation of tissue iron deposits in iron loaded liver tissue. Four liver samples reported in a previous study [17] (from the group of non-transfusion dependent β-thalassaemia/Hb E patients) gave values of F_S in the approximate narrow range 0.08 to 0.14. No data are available for transfusion dependent β-thalassaemia liver tissue. Samples of pancreas tissue from three non-transfusion dependent β-thalassaemia/Hb E patients did show a somewhat larger variation in F_S between individuals (range from 0.12 to 0.27) [17]. The coefficient of variation of χ_{Fe} for all spleen specimens studied here was 21% (somewhat larger than the average analytical uncertainty on the measurement of χ_{Fe} for an individual specimen of approximately 10%) suggesting that biological variability in χ_{Fe} would contribute a random uncertainty of approximately 19% to non-invasive measurements of spleen non-haem-iron concentrations using biomagnetic susceptometry. If the currently accepted value of χ_{Fe} for use in liver biosusceptometry (1.6×10^{-6} m^3/kg_{Fe}) was used to measure spleen iron concentration in the entire group of patients in this study, there would be a systematic under-estimation of the tissue non-haem-iron concentration of 12.5% (SE 6%).

Further measurements of χ_{Fe} for liver tissue specimens will need to be made in order to determine whether similar magnitudes of uncertainty would apply to biomagnetic susceptometry measurements of liver iron concentration. Differences in the iron-specific magnetic susceptibilities χ_{Fe} for liver tissue between different patients would explain some of the variability seen in comparisons of measurements of liver iron concentration measured by non-invasive magnetic measurements with those measured by chemical assay of biopsy specimens.

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